Reproduction in *Olivella biplicata*

BY

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(Plate 44; 3 Text figures)

IN CONJUNCTION with more general studies of *Olivella biplicata* (SOWERBY, 1825) considerable information has been obtained on its reproduction. These observations cover aspects of sexuality, the courtship and mating behavior, including documentation of a nonrandom mating pattern, evidence of year-round mating and settlement, a fecundity count, and the first description of the egg capsule, larva, and form of early development. Field work was done at various sites on the Oregon and California coasts; laboratory investigations were done at the Oregon Institute of Marine Biology, Charleston, Oregon, and at Scripps Institution of Oceanography, La Jolla, California.

GONOCHORISM

Olivella biplicata is dioecious with internal fertilization, but sexual dimorphism is not conspicuous. Sex is most easily determined from the positions of individuals in courting pairs. Alternatively presence or absence of a penis, shape of the ventral pedal gland, gonad color, or form of the genital tracts may be used. Although presence of a penis is decisive, absence may indicate either a female or a male that has lost sex characters owing to trematode infection, in which case a small, easily overlooked rudiment may remain. Females possess an inconspicuous, cup-shaped pedal gland for molding and attaching egg capsules, whereas males have a longitudinal, probably glandular (see later) slit at this site. The mature testis is orangeish, the ovary yellowish, but the difference is slight and is lacking in immature or parasitized animals. The anatomy of the genital tracts is given in MARCUS & MARCUS (1959 a) and ZELL (1955).

Unlike most prosobranchs (COMFORT, 1957; FRETTER & GRAHAM, 1964; GALTSOFF, 1961; ABBOTT, 1954; ROBERTSON, 1959), male *Olivella biplicata* grow faster

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(FRANK & EDWARDS, unpubl. data) and are larger than females. (Size data hereinafter are shell lengths from the siphonal canal to the apex.) The sexual size dimorphism was demonstrated by measuring and sexing the mature snails taken in total-count transect sampling across *Olivella*-occupied beaches (Table 1). In addition, in experiments regarding size as a treatment, randomly selected "Large" snails (22.0 - 25.0 mm) were nearly all males, whereas most of the "Medium" snails (17.0 to 20.0 mm) were females. Finally, males taken in courting pairs were significantly larger than their female partners (Table 2; Figures 1 and 2). Thus shell size and the spacing of growth lines provide weak sex indicators: lines are close together on slow-growing female shells, which rarely exceed 23 mm, but further apart on male shells, which are often large (up to 30 mm).

Table 1

Mean lengths ($\overline{X}$), standard deviations (SD), and numbers (N) of mature snails of each sex taken in transect sampling at two beach sites on the Oregon coast in 1964.

| Site | Males | | | Females | | | P |
	$\overline{X}$(mm)	SD	N	$\overline{X}$(mm)	SD	N	(t-test)
Yaquina Bay	19.55	1.57	67	18.80	1.15	45	0.01
Coos Bay	19.84	2.67	52	18.45	1.71	58	0.01

Both STOHLER (1959-1960) and I (several sets of measurements) find that, in contrast to some gastropods (FRETTER & GRAHAM, 1964), female *Olivella biplicata* shells are no more tumid than male ones.

Among mollusks generally, and including *Olivella verreauxii* (DUCLOS, 1857) (MARCUS & MARCUS, 1959 a and b), females tend to be more numerous than males. As the disparity is lacking in young animals and increases with age, it is attributed to earlier death in males (FRETTER & GRAHAM, 1964). COMFORT (1957) also reports

greater longevity for females. These findings could, how-ever, be due to mistaken sex identifications with para-sitized animals, wrongly equating age with size, or samp-ling bias favoring capture of larger individuals. Sex ratios for *O. biplicata* can be read from the N columns in Table 1. The ratio was approximately 1:1 for both whole populations and by beach levels within them. Of the 52 males (vs. 58 females) taken at Coos Bay less than half (23) had a functional penis – often it was absent. For the effective sex ratio to be unity, the probability of sexual impairment from parasitic infection would have to be the same for both sexes. At Yaquina Bay, where design forbade dissections, 67 males, 45 females, and 16 "unknowns" were taken. Most "unknowns" would be females, since their sex characters are hard to detect and positive identifications were required.

Olivella biplicata of both sexes mature sexually at about the 16 mm size: these are the smallest snails taken in numbers in courting pairs (Figures 1 and 2). Since 16 mm probably represents about 1 year's growth and *O. biplicata*'s longevity likely exceeds 10 years (FRANK & EDWARDS, unpubl. data; STOHLER, 1962, and personal communication), about 10% or less of the life span is passed in immaturity. Prosobranchs generally exhibit no genetically set post-reproductive period, though heavy trematode infections can have the same effect.

MATING BEHAVIOR

Courtship in *Olivella biplicata* is initiated by the male's extending his propodium and grasping the female's shell at or near its apex. A tandem, courting pair results, with the female in front, the male behind (Plate 44). While joined, the pair alternately moves about and sits quietly; the female may feed. A characteristic activity of courting snails is a forward-and-back rocking of the body on the large foot. This motion is more common in females, who initiate it, than in males, but at times the partners rock together in unison. Whether this activity is a necessary prelude to copulation is not known, but ZELL (1955) believes it is one step in a reciprocal reaction chain like those demonstrated in the courting sequences of many animals (TINBERGEN, 1953).

Although MARCUS & MARCUS (1959b) found no evidence for it in *Olivella verreauxii*, the bond between courting *O. biplicata* is a sticky mucus, strands of which adhere to the shell apices of females from pairs. This ad-hesive is apparently secreted by the male's pedal gland, which may, in courting males, be filled or covered with thick mucus. This connective can support the weight of the male when the female is lifted from the substrate,

and the coupling is maintained when a pair is rolled about by beach surf. In a shifting medium like sand an attach-ment between courting individuals likely helps ensure fertilization (see PEARSE et al., 1942).

Pairings may persist for a long time: one couple was joined continuously for at least 31 hours, and another paired repeatedly for 3 days. During courtship the male extends his penis along the right side of the female shell and, when successful (many attempts fail; males are very sensitive to disturbances at this vulnerable stage), into her mantle cavity. Males have sometimes advanced on the right side of the females' shells by this stage. During intromission, which on several occa-sions lasted about 10 minutes, the two snails are firmly locked together. Copulation may occur repeatedly be-tween a single pair, or individuals may mate with several partners in a few days time.

Mate-finding in *Olivella biplicata* seems to be based on males' reactions to short-lived chemicals left in female mucus trails, contact chemosensory responses, and pos-sibly tactile cues, but not on distance chemoreception. Although these snails often travel for a distance in each others' sand trails (probably a tactile response), males are unable to use direct trail following to locate distant females. But a male coming upon a very fresh track of a sexually-ready female (usually less than 15 cm away) turns into the track, accelerates, makes contact, and attempts to pair. Evidence for a chemical stimulus in female mucus trails is furnished by occasional obser-vations of triplets consisting of a regular courting pair with a second male behind the first, all linked in tan-dem. No pairings of males alone were observed. (One new male bit the courting male, who turned aside, the former taking the female!) Males who paired repeated-ly, however, showed surprisingly little ability to relocate their partner when pairing was interrupted: although lying just behind the female, they often moved off in an inappropriate direction, sometimes attempting un-successfully to attach to another nearby snail. Once (San Diego Flood Control Channel; 3 August 1966) a male *Olivella baetica* CARPENTER, 1864, was found courting a female *O. biplicata*, suggesting that initially sex may be more readily discriminated than species. During courtship, males often curl the lateral edges of their propodia anteriorly under the females, contacting the latter's metapodia; females sometimes extend their proboscides to their shell apices and the male propodia and occasionally bite the latter, terminating courtship. Females may also curl the posterior edge of the meta-podium dorsally, preventing male coupling – though one male bit this barrier and later formed a pair. These

contacts would permit contact chemosensory responses and further discrimination of partners.

Distance chemorception in mate-finding was tested on a large (91 cm by 290 cm) outdoor water table fitted with a median longitudinal divider (115 cm) at the water inlet end. On one side sea water passed through an enclosure of females from pairs and on the other through an empty enclosure. Males from pairs were released at the downstream end of the table, but showed no tendency to move preferentially toward the females. The high densities in natural *Olivella biplicata* populations and the snails' considerable mobility should produce frequent contacts between sexually ready individuals without special means of mate-finding at a distance being required.

On the gently sloping, protected sand beaches where *Olivella biplicata* usually lives, this snail develops a size class distribution with large animals higher on the shore, smaller lower (EDWARDS, 1965). The resulting graded separation of reproductives and immatures should further aid mate-finding efficiency.

Measurements of individuals in courting pairs showed that 1) males were significantly larger than their female partners, as expected from the sex difference in growth rate (Table 2 and see above) and 2) mating is non-random, larger males pairing with larger females, smaller males with smaller females (Figures 1 and 2). The best evidence for the mating pattern is given in Figure 1, since at the Charleston lagoon the lengths of 210 nearest neighbors of 97 pairs were found to be independent of the courting females' sizes: the regression slope b=0.067 was not statistically significant (data furnished by Peter W. Frank). Apparently lagoon channel shifts and currents do not permit a size class distribution to be developed at this site. Inspection of Figure 1 suggests large males mate less selectively than small males, which court only smaller females. Figure 2, on the other hand, presents data from sites where segregation of *Olivella biplicata* by size category has been demonstrated (Yaquina Bay, Coos Bay, and Duxbury Reef [EDWARDS, 1965]) or indicated (Monterey Harbor [REYNOLDS, 1948] and San Diego Flood Control Channel [F. Wolfson, cited in STOHLER, 1959-1960]). Although the relationship between male and female sizes is not statistically significant for any of these small samples, the trend is evident: the regression line for the grouped data (N=82) is

$$Y = 13.223 + 0.444 \, X,$$

slope b statistically significant at the 0.01 level. The steeper slope for these samples, compared to the Charleston lagoon value (b=0.350), may be due to the size class distribution. Also at Duxbury Reef, where only larger animals were taken in pairs (Figure 2), the nonselectivity of large males is clear (nonsignificant slope b=−0.343).

Although differences in activity cycles – large snails are active at night, whereas small ones may be active in the light (EDWARDS, 1965) – could account for non-random mating, the nonselectivity of large males sug-

Table 2

Differences in lengths of males and females taken in courting pairs at six sites on the Oregon and California coasts.

Site	Date	Pairs (N)	Pairs with ♂♂ > ♀♀	Mean Difference ♂−♀ (mm)	SD	p[A]
Yaquina Bay (Tide pool stream)	June-July 1964	18	17	2.88	2.53	0.001
Coos Bay (Charleston lagoon)	July-Aug. 1963	197	150	1.85	2.53	<0.001
Coos Bay (Beach)	June-July 1964	20	15	1.94	2.77	0.01
Duxbury Reef[B]	Oct.-Jan. 1964-65	22	18	2.51	2.43	0.001
Monterey Harbor[c]	Nov. 1964	6	3	0.22	1.96	0.80
San Diego Flood Control Channel	Oct.-June 1965-66	16	14	1.91	2.31	0.01

[A] tested by paired comparison t-test
[B] STOHLER (1959, 1960) found females larger than males in all of 11 pairs taken here. No explanation for the disagreement is available except for sampling error
[c] no large snails were taken in pairs here (cf. Text figure 2), possibly owing to trematode infections

gests another explanation. Since in mating a male must extend his penis most of the length of the female's shell and into the mantle cavity, probably small males simply
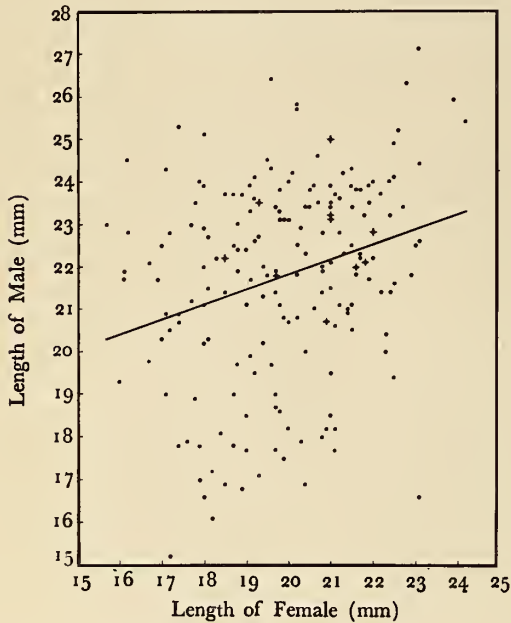

Figure 1

The relationship between lengths of males and females taken in 197 courting pairs at the tidal lagoon, Charleston, Oregon, July to August 1963. Single pairs are indicated by a solid circle, two pairs by a circle with a cross through it. The regression line, fitted by the least squares method, is $Y = 14.815 + 0.350 X$. Slope b is statistically significant at the 0.001 level. (Data collected by Peter W. Frank)

cannot accommodate larger females. A suggestion that small males are at a disadvantage in courting comes from a comparison between mean sizes of mature animals taken in total count sampling and those from courting pairs: on the Coos Bay beach, the only site for which such data are available, courting males were somewhat larger than mature males over-all (20.78 mm vs. 19.84 mm; P = 0.10), whereas female sizes were very similar (18.84 mm vs. 18.45 mm; P = 0.50). Another test would be a comparison of the mating success of large females isolated with large vs. small males. If small males are inferior mating partners, the size class distribution of *Olivella biplicata* would promote efficient pairing both by separating mature snails from immatures and by placing large matures with large, small with small. The more rapid growth of males may also be related to their

mating effectiveness. Since pairing data came from courting snails, many of whom had likely not yet copulated, apparently either or both sexes can recognize and select, probably by chemosensory means (see above), partners of suitable sizes before actual mating attempts.

YEAR-ROUND MATING
AND SETTLEMENT

Three lines of evidence indicate that *Olivella biplicata* both breeds and spawns all year. (1) STOHLER (1959-1960) and I have both observed courting pairs in every month. Numbers mating at a given time seem to be due more to as yet undetermined local, short-term conditions than to annual changes: large numbers mate in every season, though on successive spring tide series pairs may be abundant or scarce. (A complicating factor is that pairs are easier to find in dim light and quiet water.) (2) The smallest, youngest snails taken by sieving (4 to
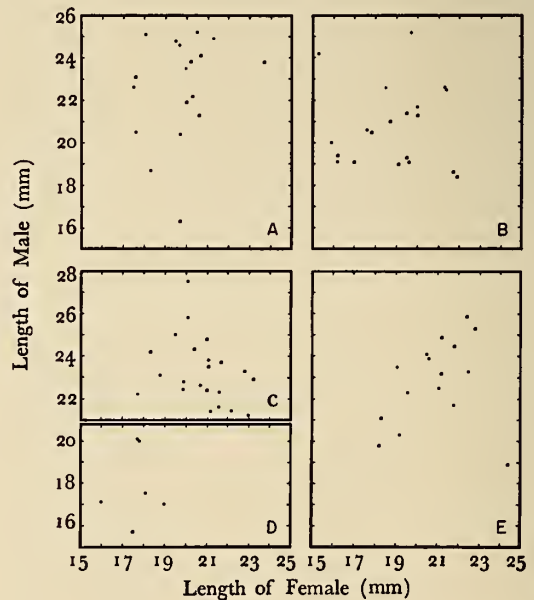

Figure 2

The relationship between lengths of males and females taken in courting pairs at five sites on the Oregon and California coasts.
(A) Yaquina Bay (44° 37′ N Latitude)
(B) Coos Bay, beach (43° 21′ N Latitude)
(C) Duxbury Reef (37° 54′ N Latitude)
(D) Monterey Harbor (36° 36′ N Latitude)
(E) San Diego Flood Control Channel (32° 45′ N Latitude)
Sampling dates are given in Table 2

A courting pair of *Olivella biplicata* with the female in front
and the male following

5 mm) entered the Coos Bay beach population through-out the year and occurred at every locality on every sampling date. (3) Size frequency distributions for populations showed no breaks that would indicate year classes. Because small animals do not cease growing in winter as mature snails do (Edwards, unpubl.), the lack
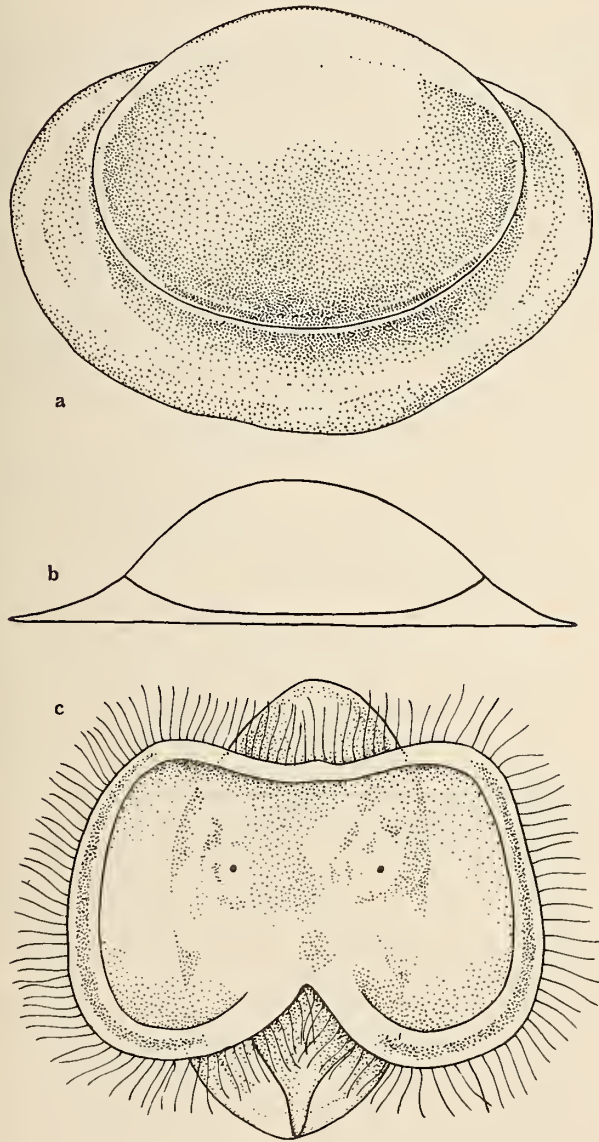

Figure 3

(A) The egg capsule of *Olivella biplicata*; three quarters view from above. (B) The same; diagrammatic cross section. (C) The veliger larva of *Olivella biplicata* shortly after hatching. These sketches of semi-transparent materials are not accurate in details.
(Drawings by Susan Ahrend)

of year class modes cannot be accounted for on the basis of mating and spawning only occurring during periods of growth for all snails. Whether individuals, in contrast to populations, exhibit reproductive cycles is not known.

Using the occurrence of mating pairs or of very small animals at different times and places, Gifford & Gifford (1942, 1944, 1948) proposed a complicated series of mating seasons for *Olivella biplicata*. Their data equally well support year-round reproduction.

Year-round reproduction occurs in a number of marine gastropods (Moore, 1938; Ricketts & Calvin, 1962; Thorson, 1950). In *Olivella biplicata* continuous breeding may be related to the genus' neotropical origins, there being some evidence that tropical forms enjoy longer breeding seasons than temperate ones (Thorson, 1950). However, *O. mutica* (Say, 1822) in Florida apparently spawns for only about one month in spring (Paine, 1962), and *O. fulgurata*'s (A. Adams & Reeve) spawning season in Japan is May to July (Habe, 1960).

Unless having new young enter the population all year is crucial for *Olivella biplicata*'s success, the factor thought to limit northward ranges of species, and hence faunal provinces, viz. the continuous period that sea temperatures meet requirements for reproduction and early growth (Hall, 1964), does not seem applicable here. Yet *O. biplicata*'s species range – Magdalena Bay, Baja California, Mexico (25° N Lat.) to lower Vancouver Island (49° N Lat.) (Keen, 1937) – closely fits the limits of the Oregonian plus the Californian shallow water, marine faunal provinces (Keen, 1958; Hall, 1964) [2].

EGG CAPSULE, FECUNDITY
AND TYPE OF EARLY DEVELOPMENT

Olivella biplicata's egg cases and form of development were found by collecting courting females in the San Diego Flood Control Channel and maintaining them at Scripps Institution of Oceanography in aquaria provided with running sea water (14° to 15° C), a sand substrate, and possible ovipositing sites. Egg capsules were subsequently found on various bivalve shells, empty *O. biplicata* shells, glass slides, and glass and plastic dishes, but not on the mother shells as reported for Japanese species (Habe, 1960; Horikoshi, unpubl.). As in *O. mutica*, *O. pusilla* and *O. verreauxii*, empty shells are likely the substrate used by *O. biplicata* in nature. Capsules are attached separately and preferentially in depressions or grooves (e. g. on *Donax*); their dispersion pattern on

[2] I. McT. Cowan (1964, personal communication) has, however, found empty shells of *Olivella biplicata* as far north as Queen Charlotte Island.

shells is markedly contagious, and on a smooth surface several may be placed contiguously.

The egg capsule (Figures 3 a and 3 b) consists of a dome-shaped cap with an indistinct median suture (not shown in the Figures) above a broader, slightly irregular base. Capsules are small (0.5 mm basal diameter; 0.3 mm lumen diameter), transparent, and unsculptured. A fission line borders the convex cap, which often comes free of the base at hatching. The capsule appears very similar to that figured for *Olivella fulgurata* from Japan (AMIO, 1963), but differs slightly from that of *O. verreauxii* from Brazil (MARCUS & MARCUS, 1959b), whose cap bears sculptured ridges, and considerably from those of *O. mutica* (PAINE, 1962) and *O. pusilla* (MARRAT, 1871) (PERRY & SCHWENGEL, 1955) from Florida, whose caps are more apical with a ridged border.

Although early development was not studied, *Olivella biplicata* would be good material for such work. Each transparent egg capsule contains one clearly visible white egg or embryo without "nurse eggs." Cleavage and larval stages are distinctly observable. The snails will oviposit on glass slides, and the time of spawning can be known precisely by exposing clean slides to mated females for short time intervals. Finally, year-round reproduction means eggs and larvae would be available in large numbers all year.

Numbers for eggs laid in capsules are generally modest compared to those broadcast into the sea. *Olivella* further provides for its young by placing them one to a capsule. To determine fecundity, courting females from the field were placed in separate clear plastic dishes provided with screen tops and running sea water (14° to 15°C). The containers were periodically examined for capsules, the females being transferred to clean dishes. One effort was successful. By total-count a 20.6 mm female deposited 4 236 egg capsules in 46.8 days (June 22 to August 8, 1966); mean per day rate was 90.5 (range 60.6 - 171.4 for 3 censuses). This is a minimum estimate, since spawning may have ceased due to depletion of the gonad, decline in the female's vigor under artificial conditions (occasionally algal food was supplied), or lack of opportunity for repeated mating. The female may also have mated and spawned before capture. This count lies among previously reported values for capsule-spawning snails (PELSENEER, 1935). Size specific fecundity data would be valuable, as they might indicate the adaptive value of large size, slow growth, and long life in *O. biplicata* (cf. MCLAREN, 1966).

Development time in the capsule was highly variable. Many young hatched by day 10, but others were still alive in their capsules after 28 days. All capsules were kept in 14° to 15°C circulating sea water, but the late-hatching animals were subjected to occasional warming during microscope observations.

The young emerge as veligers (Figure 3 c). The shell is nearly transparent and unsculptured; it is a barely skewed helix of ca. 1⅓ whorls and a maximum diameter of 0.2 - 0.3 mm. The large, bilobate velum has a light brown band of pigment near its borders and bears eye spots on slight prominences anteriorly. The body is nearly colorless, and internal organs, especially the visibly beating heart, are easily seen. The operculum is clear and scalelike.

The veligers are apparently nonpelagic. Rather they swim, anterior (eye spots) end down, at the bottom of their containers, opening and closing their velum lobes against the substrate. Occasionally they swim in a tight vertical loop, but return quickly to the bottom. The larvae appear to be examining the substrate. When clean sand was added to a dish, the animal opened and closed its velum around several grains, then swam on. Possibly these larvae never join the plankton; but, perhaps responding negatively to light and positively to gravity (several tests proved inconclusive), swim at the bottom until suitable cues for metamorphosis – presumably ones associated with lower shore situations – are detected. Balanced sex ratios across beach levels show females do not move down shore to spawn. The larval behavior would explain how the young, hatching higher on the shore where adults live, are able to cross wide expanses of beach and reach the lower shore levels where physiological stresses are within their ranges of tolerance (EDWARDS, 1965).

No larvae metamorphosed – or developed appreciably – when maintained in bare glass or plastic dishes, or in ones provided with sand. Isolated individuals died after 3 - 4 days. Presumably stimuli required for metamorphosis were missing from the laboratory set-up. Aggregations of very small snails occur on the shore, suggesting either that settlement is stimulated by irregular features of the environment or presence of settled young, or that metamorphosed young show strong positive responses to one another.

Since close relatives of *Olivella biplicata* from warmer water exhibit either direct development – *O. fulgurata* (AMIO, 1963), *O. spreta* GOULD, 1860 (HORIKOSHI, unpubl.), *O. mutica* (PAINE, 1962), and *Ancilla* sp. (NATARAJAN, 1957) – or a short, nonpelagic larval life – *O. verreauxii* (MARCUS & MARCUS, 1959b) – *O. biplicata* would have been expected to show direct development in cool West Coast waters (THORSON, 1950). Long breeding seasons are also often linked to direct development (THORSON, op. cit.). Yet the mixed occurrence, even within genera, of species with free-swimming veligers and others with direct development is well-known